

Supplemental information

Rational discovery of therapeutic PAK1

allosteric activators

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Rational Discovery of Therapeutic PAK1 Allosteric Activators

Yu He (何钰)^{1, #}, James S. H. Bae^{1, #, ☒}, Elżbieta Nowak^{2, 3}, Carlos Outeiral⁴, Daniel A. Nissley⁴, Anthony Tumber⁵, Georgina Berridge⁶, Eidarus Salah⁵, Yi Wang⁷, Wenqi He⁸, Hongyuan Zhang⁸, Tangting Chen⁹, Samuel Tusk^{10, 11}, Sebastian Mathea^{12, 13}, Ying-Jie Wang¹⁴, Alexander Grassam-Rowe¹, Philipp Kukura^{10, 11}, Christopher J. Schofield⁵, Darragh P O'Brien⁶, Andrea Pierangelini⁶, Grant C. Churchill¹, Thomas Lanyon-Hogg¹, Yunbo Ke¹⁵, Chao Xu¹⁶, Tao Ye^{16, 17}, Hugh Watkins¹⁴, Liming Ying¹⁸, Andreas Koschinski¹⁹, R. John Solaro²⁰, Xiaoqiu Tan⁹, Jani R. Bolla⁷, Xin Wang⁸, Stefan Knapp^{12, 13}, Charlotte M. Deane⁴, Manuela Zaccolo¹⁹, Marcin Nowotny², Ming Lei^{1, ☒, *}

Table S1. X-ray data collection and refinement statistics (PAK1 kinase domain with NVS-PAK1-1 inhibitor), related to Figure 3 and STAR Methods.

Data collection	
Beamline	14.1 BESSY
Space group	$P2_1$
Cell dimensions	
<i>a</i> , <i>b</i> , <i>c</i> (Å)	63.6, 75.4, 65.1
α , β , γ (°)	90.0, 107.8, 90.0
Resolution (Å)*	37.9 - 2.28
R_{merge} *	0.161 (1.66)
$I / \sigma I$ *	10.5(1.0)
$CC_{1/2}$ *,**	99.7 (39.4)
Completeness (%)*	97.8 (92.9)
Redundancy*	6.5 (5.7)
Refinement	
Resolution (Å)	37.9-2.28
No. reflections	26282
R_{work} (%)	21.75
R_{free} (%)	26.55
No. atoms	
Protein	4241
Ligand/ion	41
Water	185
<i>B</i> factors (Å ²)	
Protein	49.6
Ligand/ion	43.0
Water	44.8
Root mean square deviations	
Bond lengths (Å)	0.006
Bond angles (°)	0.896

The data collection statistics are based on a single crystal. *Values in parentheses are for highest-resolution shell. ** $CC_{1/2}$, correlation coefficient between the average intensities in two parts of the unmerged data, each with a random half of the measurements of each unique reflection [Karplus PA, Diederichs K. 2012. Linking crystallographic model and data quality. *Science* 336:1030-3].

Table S2. Summary of HDX-MS experimental conditions for PAK1 in apo and holo states with or without PAK1-A1, related to Figure 5 and STAR Methods.

Data Set	PAK1 (apo)	PAK1 + PAK1-A1
HDX reaction details	50 mM TRIS pH 6.42, 150 mM NaCl, 1 mM TCEP, pD = 6.40, 20 °C	50 mM TRIS pH 6.42, 150 mM NaCl, 1 mM TCEP, pD = 6.40, 20 °C
HDX time course (Corrected for pH 7.5)	1, 3, 6, 600 (sec)	1, 3, 6, 600 (sec)
HDX control samples	N/A	N/A
Back-exchange (mean / IQR)	N/A	N/A
# of Peptides	85	85
Sequence coverage	91.1%	91.1%
Average peptide length / Redundancy	16.8 / 2.5	16.8 / 2.5
Replicates (biological or technical)	1 sec n = 3; 3 sec n = 1; 6 sec n = 5; 600 sec n = 3	1 sec n = 3; 3 sec n = 1; 6 sec n = 4; 600 sec n = 2
Repeatability	0.0721 (average standard deviation)	0.0567 (average standard deviation)
Significant differences in HDX (delta HDX > X D)		0.20 D (95% CI)